



Research Article

Chromatographical Analysis of Amino Acids in Sepia Mutant of *Drosophila melanogaster* under Stress of Cypermethrin and Alphamethrin

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Abstract: Insects are invertebrates that are taxonomically referred to as the class Insecta. They are the most numerous and most widespread terrestrial taxon within the phylum Arthropoda, and indeed the most diverse group of animals on the earth, with around 925,000 species described—more than all other animal groups combined. Insects may be found in nearly all environments on the planet, although only a small number of species have adapted to life in the oceans where crustaceans tend to predominate instead. HPTLC is a method commonly applied for the identification, assay and the testing for purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutrients). These flexible and cost-effective techniques present the advantage of the simultaneous processing of standards and samples with versatile detection possibilities, including a great variety of post-chromatographic derivatization reagents.

Keywords: HPTLC, Leucine, Glycine, Valine, Marker compounds, Pyrethroid insecticides.

1. Introduction

There are approximately 5,000 dragonfly species, 2,000 praying mantis, 20,000 grasshoppers, 170,000 butterflies and moth, 120,000 fly, 82,000 true bug, 350,000 beetle, and 110,000 bee and ant species described to date. Estimates of the total number of current species, including those not yet known to science, range from two million to fifty million, with newer studies favouring a lower figure of about six to ten million. Adult modern insects range in size from 0.139mm (*Dicopomorpha echmepterygis*) to 555mm (*Phobaeticus serratipes*).

The use of chlorinated and organophosphate insecticides in the household is being discouraged throughout the world. These insecticides are considered to have high persistence, adverse environmental impact and toxic effects on higher animals including man. On the other hand, in the past decade, the synthetic pyrethroids have emerged as a major class of agriculturally useful organic insecticide. Pyrethroids are a diverse group of chemicals, which have been developed to kill or suppress a wide variety of insect pests. Application of pyrethroids and other insecticides

for protection against infestation is a successful approach to the problem of preventing damage to crop seeds and keeping grains free from insects (Tufail *et al.*, 1992). Pyrethroids are synthetic derivatives of natural pyrethrum which is obtained from the flower heads of *Chrysanthemum cinerariaefolium* and some other species of *Chrysanthemum*. Natural pyrethrum and synthetic pyrethroid insecticides are considered among the safest class of insecticides available.

The insecticidal activity of pyrethrum is due to six esters namely pyrethrum I, II; cinerin I, II and jasmolin I, II. Natural pyrethrins are light sensitive and undergo rapid photodegradation, while synthetic pyrethroids are photostable i.e. they do not undergo photolysis in sunlight. Pyrethrum is a complex insecticide with the following outstanding properties:

a. Rapid Action: Pyrethrum is a contact insecticide attacking the nervous system of insects almost immediately and causing knockdown, soon followed by kill.

b. Low Mammalian Toxicity: No other insecticide can claim such a long record of proven safety towards humans and warm blooded animals. It is one of the

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least toxic of domestic insecticides available and is rapidly metabolized if accidentally swallowed.

c. Lack of Insect Immunity: The structure of the molecule is so complex that insect resistance to pyrethrum is not a practical problem. A very few isolated cases have been reported, unlike synthetic insecticides.

d. Broad Spectrum of Activity: Because pyrethrum consists of a group of related compounds, it has a wider spectrum of activity against insect species than many single insecticides. It can be used against any insect found in the house or garden: mosquitoes, flies, cockroaches, aphids, ants.

e. Environment friendly: Nonresistant: Pyrethrum is degraded by the combination of sunlight and air and therefore presents little of the hazards which are usually associated with other classes of persistent insecticide.

f. Repellency: Pyrethrum is a powerful insect repellent which in combination with its low mammalian toxicity favors it in many applications, such as food and grain storage protection, personal protection (spray-on, roll-on, mosquito coils). Beside repellency, mosquito coils will inhibit target insects from biting.

g. Flushing Action: Pyrethrum has a much greater flushing effect than any other insecticide; it disturbs pests in their hiding places, forcing them to get out and to get exposed to the insecticide.

HPTLC is the method commonly applied for the identification, assay and testing for purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated product (pharmaceuticals, cosmetics, nutrients). This flexible and cost effective technique presents the advantage of the simultaneous processing of standards and samples with versatile detection possibilities, including a great variety of post chromatographic derivatization reagents. The validation of analytical methods is largely recognized as the best safeguard against the generation of unreliable data and is becoming an absolute requirement in many fields. Validation is the process by which it is established, by laboratory studies, that the performance characteristics of an analytical method meet the requirements for the intended application. HPTLC is finding increasing use in various fields today. The development of scanning, densitometry and derivatization have also helped in making it more useful. Alteration in HPTLC of treated sets is an index of toxicity so that the outcome of present investigation becomes fruitful and genetically evidenced.

2. Materials and Methods

2.1 Laboratory culture of experimental mutants

The Sepia mutant form of *Drosophila melanogaster* was acquired from toxicology laboratory, Department of Zoology, Dr. B.R. Ambedkar

University, Agra. The culture was nurtured and maintained in glass culture vials of 100ml capacity, at a temperature $25 \pm 0.5^{\circ}\text{C}$, $50 \pm 5\%$ relative humidity in B.O.D. incubator. The flies were fed on prescribed *Drosophila* food (Roberts, 1986).

2.2 Experimental protocol

For the chromatographical analysis experiment was conducted on three sets of Sepia mutant of *Drosophila melanogaster*.

- Control set;
- Cypermethrin treated set and;
- Alphamethrin treated set.

First set was free from treatment and considered as control set, while the second and third sets were given sublethal treatments of cypermethrin and alphamethrin respectively for 48 hours and served as treated sets. Flies of all the sets were allowed to fertilize for five days and the observation was made in the F_1 generation.

3. Result and Discussion

An increase has been observed in area percentage of cypermethrin and alphamethrin treated sets as compared to control set. However, the increase is more in alphamethrin treated sets as compared to cypermethrin treated sets (Table 1-3; Fig. 1-4).

Table 1. TLC data of glycine amino acid in sepia mutant of *Drosophila melanogaster*.

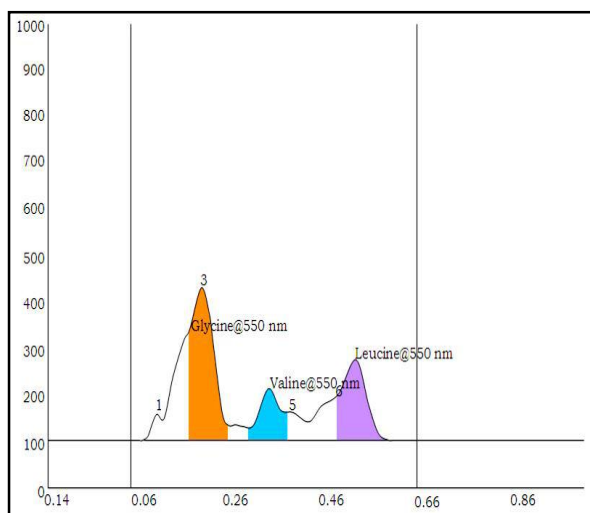
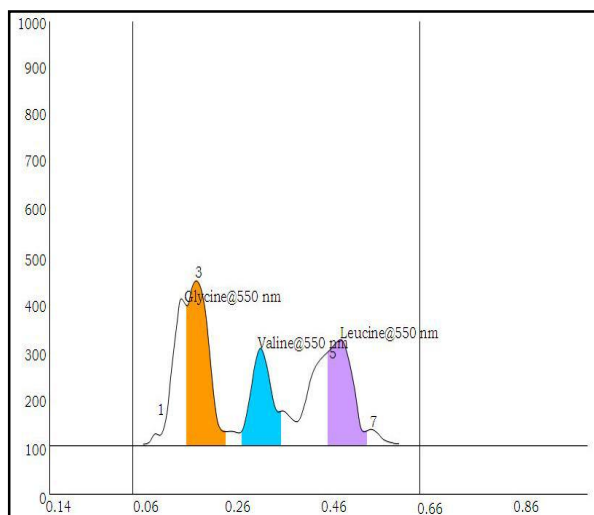
Parameters	Control	Treated	
		Alphamethrin	Cypermethrin
Start R_f	0.10	0.09	0.09
Start height	41.8	17.4	25.2
Max ^m R_f	0.16	0.15	0.14
Max ^m height	240.6	228.7	329.0
Max ^m %	22.22	22.68	23.34
End R_f	0.16	0.15	0.15
End height (mm)	240.6	228.7	307.8
Area (mm ²)	6549.7	5478.3	8436.8
Area percentage	18.34	17.26	17.82

Table 2. TLC data of valine amino acid in sepia mutant of *Drosophila melanogaster*.

Parameters	Control	Treated	
		Alphamethrin	Cypermethrin
Start R_f	0.28	0.27	0.27
Start height	28.3	25.0	26.6
Max ^m R_f	0.33	0.31	0.32
Max ^m height	114.2	128.4	213.9
Max ^m %	10.55	12.73	15.18
End R_f	0.36	0.35	0.36
End height (mm)	60.3	42.5	75.0
Area (mm ²)	4331.0	4448.6	7835.3
Area percentage	12.13	14.02	16.55

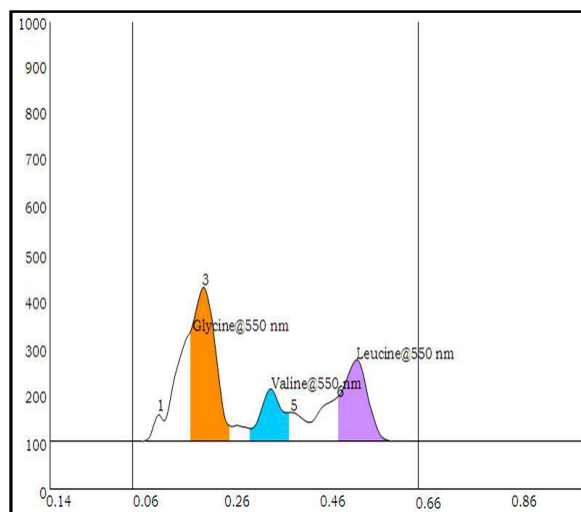
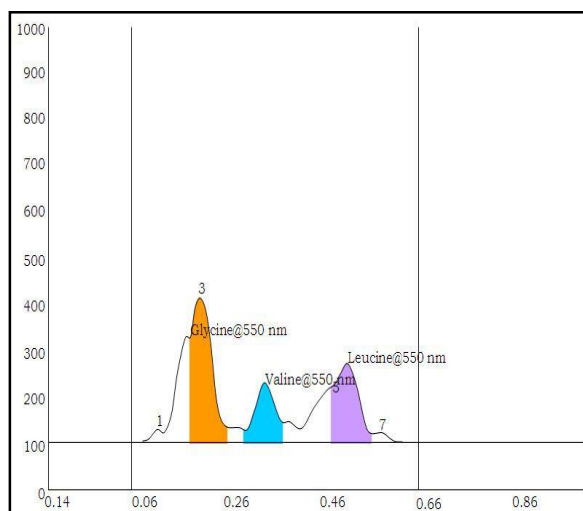
Table 3. TLC data of leucine amino acid in sepia mutant of *Drosophila melanogaster*.

Parameters	Control	Treated	
		Alphamethrin	Cypermethrin
Start R_f	0.47	0.46	0.47
Start height	105.3	119.3	203.3
Max ^m R_f	0.51	0.49	0.49
Max ^m height	178.9	172.8	233.2
Max ^m %	16.52	17.14	16.54
End R_f	0.59	0.54	0.55
End height (mm)	2.0	16.3	25.6
Area (mm ²)	7191.6	6605.0	9338.5
Area percentage	20.14	20.81	19.72

**Fig. 1. Chromatogram showing glycine, valine and leucine amino acids in control set of sepia mutant.****Fig. 2. Chromatogram showing glycine, valine and leucine amino acids in alphamethrin treated set of sepia mutant.**

The validation of analytical methods is largely recognized as the best safeguard against the generation of unreliable data and is becoming an absolute requirement in many fields. Validation is the process by which it is established, by laboratory studies, that the

performance characteristics of an analytical method meet the requirements for the intended applications. Depending on the objective of the analytical procedure, the typical validation characteristics which can be considered through a statistical approach are accuracy, precision, specificity or selectivity, detection limit, quantification limit, linearity and ruggedness. The concept of validation applied to densitometric determinations on high-performance thin-layer chromatography (HPTLC) indeed varies according to the goal of the analysis and the steps required for a formal validation have been thoroughly investigated, notably for purity testing, pharmaceutical dosage forms assay and herbals fingerprinting (Biringanine *et al.*, 2005).

**Fig. 3. Chromatogram showing glycine, valine and leucine amino acids in control set of sepia mutant.****Fig. 4. Chromatogram showing glycine, valine and leucine amino acids in cypermethrin treated set of sepia mutant.**

For standardization of natural product drugs, single chemical entities, “marker compounds,” may be used as potency standards in high-performance thin-layer

chromatography (HPTLC) analysis. Using well-characterized marker compounds, conventional pharmaceutical manufacturing criteria for assay and content uniformity may be applied. These marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes, and obtain stability profiles. HPTLC analysis of marker compounds may provide additional information in the form of "chromatographic fingerprints" (Natalie *et al.*, 1998).

Densitometric scanning of individual or all fractions can be repeated with the same or different parameters, since all fractions remain stored on the plate, and have many options for evaluating or documenting the fractions both before and after derivatization. Many samples are analysed side by side and due to the offline character of the method many samples can be chromatographed simultaneously (Janchen, 1987).

HPTLC is finding increasing use in various fields today. The development of scanning, densitometry and derivatization have also helped in making it more useful.

HPTLC was carried out for qualitative estimation of experimental amino acids. The sequence of area percentage of experimental amino acids in control sets (control sepia and control white) was found to be-

Leucine > Glycine > Valine

The sequence of area percentage remained same for the treated mutants except for alphamethrin treated white mutants in which it changed to-

Glycine > Leucine > Valine

Area percentage of glycine decreased, whereas increased of valine in sepia mutants following alphamethrin and cypermethrin intoxication, but area percentage of leucine increased in alphamethrin treated sepia mutant which decreased in cypermethrin treated sepia mutants.

The area percentage decreased of both valine and leucine amino acid in alphamethrin as well as cypermethrin intoxicated white mutants whereas area percentage of glycine increased in alphamethrin treated white mutants and decreased in cypermethrin treated white mutants.

Fluctuation in area percentage is more for valine amino acid in the two experimental mutants, whether treated with cypermethrin or with alphamethrin. Therefore, valine, an amino acid, was affected the most among the three amino acids.

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